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A NEW DESCRIPTION OF *SYNDESMIS DENDRASTRORUM* (PLATYHELMINTHES, TURBELLARIA), AN INTESTINAL RHABDOCOEL INHABITING THE SAND DOLLAR *DENDRASTER EXCENTRICUS*

NOEL S. SMITH

Department of Developmental and Cell Biology, University of California, Irvine, Irvine, California 92644

The genus *Syndesmis* was erected by Silliman (1881) for a rhabdocoel inhabiting the sea urchin *Echinus sphaera*. Describing a rhabdocoel in the urchin *Strongylocentrotus franciscanus*, Lehman (1946) defined yet another genus, naming the particular species *Syndisyrinx franciscanus*. Marcus (1949) suggested that *Syndisyrinx* was synonymous with the *Syndesmis* of Silliman (1881) and Stunkard and Corliss (1951) concurred with this view in their revision of the family Umagillidae Wahl. In this revision three species of *Syndesmis* were recognized: *S. echinorum* inhabitng several European sea urchins; *S. franciscana* (Lehman, 1946); and *S. antillarum* inhabiting Diadema antillarum (which was originally reported but not named by Powers in 1936). Also presented was a description of a fourth species, *S. dendrastrorum*, a newly discovered rhabdocoel inhabiting the sand dollar *Dendraster excentricus* at La Jolla, California.

Knowledge of the genus *Syndesmis* Silliman, 1881, was extended by Jennings and Mettrick (1968) who undertook nutritional and chemical studies on *Syndesmis franciscana* occurring in the Jamaican echinoid, *Lytechinus variegatus*. From similar studies on *S. franciscana* occurring in *Strongylocentrotus franciscanus*, Mettrick (1969) concluded that the Caribbean syndesmid was probably *S. antillarum*. Failing to cite Mettrick (1969), Jones and Canton (1970) reported additional studies undertaken on the distribution of *S. franciscana* in *L. variegatus* in the Caribbean.

In the original description of *Syndesmis dendrastrorum* Stunkard and Corliss, 1951, many of the internal organs were not observed or described. In addition, it was not determined whether the worms were found in the intestine or the coelomic cavity. The illustration presented in their description is probably not representative, as it was based on but two specimens, one of which was contorted. The purpose of my work was to determine the location and occurrence of the worm in the host echinoid and to clarify the internal relationships of the reproductive organs. Also contained in this report is the first record and description of juvenile stages of *Syndesmis*.

Materials and Methods

Collection and maintenance of hosts

Specimens of *Dendraster excentricus* were collected off Shaw's Cove at Laguna Beach, California, during the months of November, December, January, February,

and March of 1971–72. Specimens were obtained in twenty to thirty feet of water with the aid of scuba equipment. The sand dollars were immediately placed in a sea water aquarium after which they were maintained in a tank equipped with running sea water. No attempt was made to feed the organisms, however specimens were observed to feed on algae growing on the walls of the holding tank.

Examination of hosts for presence of worms

The location of the rhabdocoel in the host echinoid was determined by examining both fixed and live specimens of *Dendraster*. Sand dollars were fixed by placing them in 80 per cent alcohol for 24 hours. Sand dollars were dissected by carefully cutting out a disc of the aboral surface of the test, approximately 4.5 cm in diameter. This disc was carefully lifted up to reveal the internal organs.

The coelomic cavity was examined under a dissecting microscope for the presence of worms. Samples of coelomic fluid were also examined by flushing out the test with sea water by means of a pipette. These washings were collected and observed under both a dissecting and compound microscope. Examination of the intestine was accomplished by removing it from the organism and placing it in a petri dish of sea water. In the petri dish the lumen of the intestine was systematically examined with the aid of dissecting needles under a dissecting microscope. The location, number of worms found, and the diameter of the test (measured across the widest part on the oral surface) were recorded for each host examined. Live hosts were examined for the presence of worms within a week after collection.

Microtechnique

Following fixation in formal-alcohol (Humason, 1967), worms were prepared either as whole mounts (60 specimens) or for sectioning (25 specimens). Whole mounts were stained with Grenacher's Borax Carmine or Harris' Hematoxylin (Humason, 1967). For sectioning, specimens were embedded in Paraplast (Sherwood Medical Industries, Inc.) at 65° C, sectioned serially at 8 and 10 microns, and stained with Mallory's triple stain (Humason, 1967). Both whole mounts and sections were mounted with Histoclad (Clay-Adams, Inc.).

The slides were examined using brightfield or phase contrast illumination with a Zeiss photo-microscope. Live worms were examined using phase contrast, brightfield, and Nomarski interference contrast microscopy. Composite drawings were prepared from living material, whole mounts, sections, and enlarged photographs of representative preparations.

Results and Discussion

Previous work (Stunkard and Corliss, 1951) left unanswered whether *S. dendrastrorum* occurs in the intestine or the coelomic cavity. The dissection of both the fixed and live hosts in this study, however, revealed that *Syndesmis dendrastrorum* occurs in the intestine. Worms were found to occur from the level of the esophagus throughout the intestine to the rectum. From the examination of 23 sand dollars, ranging from 5.8 cm to 7.5 cm in diameter, a range of from 0 to 24 worms per host was observed.

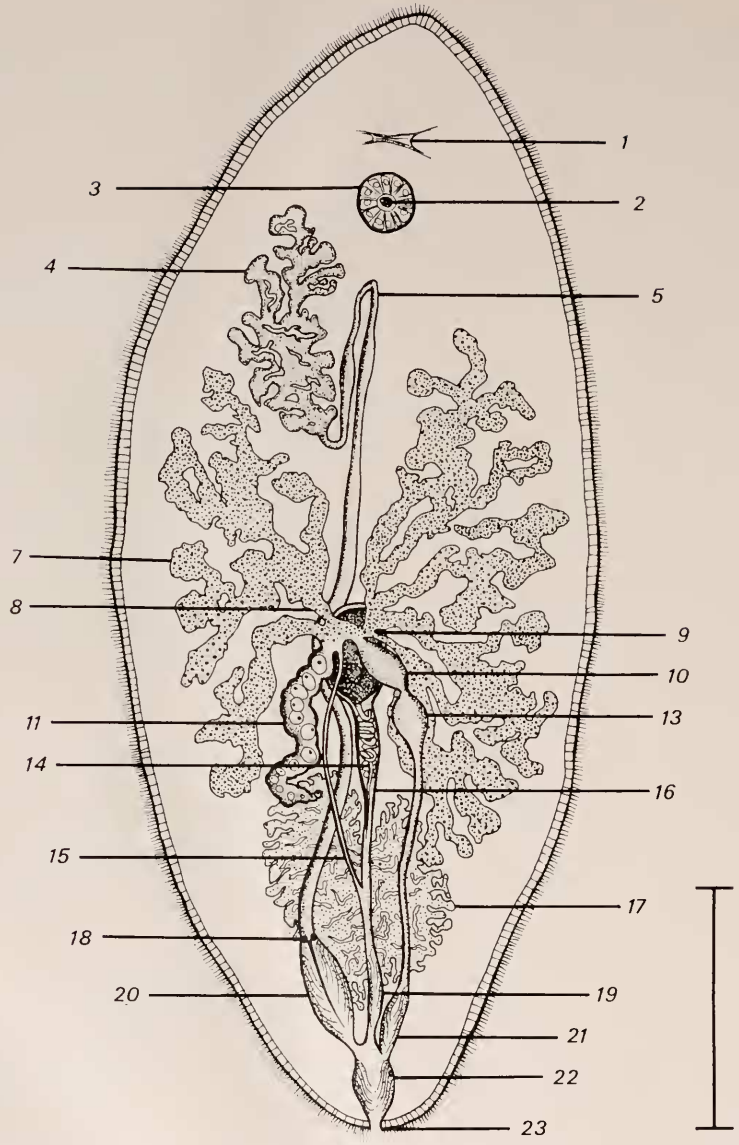

FIGURE 1. Dorsal view of *Syndesmis dendrastrorum* Stunkard and Corliss, 1951, with intestine omitted. Scale bar equals 200 microns; 1, brain; 2, buccal cavity; 3 pharynx; 4, testes; 5, sperm duct; 6 intestine; 7, vitellaria; 8, vitelline ducts; 9, egg capsule; 10, seminal receptacle; 11, ovary; 12, ova; 13, seminal bursa; 14, whip of egg capsule; 15, ovovitelline duct; 16, uterus; 17, cement glands; 18, penis; 19, female antrum; 20, male antrum; 21, bursal canal; 22, genital antrum; 23, genital pore.

As the earlier description (Stunkard and Corliss, 1951) was based on only two specimens, an incomplete account of the morphology was presented. A more detailed account follows, based on material collected at this new location. Living

specimens of *Syndesmis* are opaque white, almost colorless, with extensive vitellaria and a large egg capsule. The worms are oval, slightly pointed at the anterior and posterior ends, widest laterally at the middle of the body; they are flattened dorsoventrally, with the dorsal side slightly convex (Figs. 1 and 2). Measurements of 25 mature specimens ranged from 0.52 mm to 1.23 mm in length; the width varied from 0.26 mm to 0.66 mm. Thickness measured about 30 microns at the edges to 90 microns at the thickest point.

The entire surface of the worm is covered with a ciliated epithelium. The cilia average 6 microns in length, compared to 3 microns as reported earlier (Stunkard and Corliss, 1951). The epithelial cells are about 6 microns in thickness and are irregular in shape.

The musculature and parenchyma is essentially the same as reported by Stunkard and Corliss (1951), and as reported for *Syndesmis franciscana* (Lehman, 1946). The epithelial cells are bounded inside by a basement membrane, under which lies a layer of circular, epithelial muscles. Under the circular muscles lie separated longitudinal fibers which occasionally transverse the parenchyma and are attached to the internal organs. The parenchyma, as in most Platyhelminthes, fills the spaces between the internal organs and the subepithelial muscles. It consists of large, irregularly shaped, vacuolated cells.

The brain is typical of previously described species. It is located approximately one-third of the way between the pharynx and the anterior end (Figs. 1 and 2). It consists of a nerve commissure connecting two ganglia.

An opening in the ventral epithelium approximately one-sixth of the distance from the anterior end of the worm forms the mouth. Immediately dorsal to the mouth is a buccal cavity surrounded by cells continuous with the epithelium. Connected dorsally to the buccal cavity is a doliiform pharynx, characteristic of the family Umagillidae, which averages approximately 54 microns dorsoventrally and 56 microns at its widest point (Figs. 1 and 2). The musculature of the pharynx follows that of *S. franciscana* (Lehman, 1946). The lumen of the pharynx is encircled by layers of distinctly staining vertical and circular muscle fibers. Radial fibers, extending from the lumen to the periphery of the pharynx, are also present with darkly staining cells filling the spaces between them (Figs. 1 and 2).

The esophagus opens into the intestine and extends dorsally from the lumen of the pharynx. As reported by Stunkard and Corliss (1951), the intestine lies directly under the dorsal epidermis, is medial in its position and extends from the pharynx to the level of the egg capsule (Fig. 2). The intestine is narrow and tubular, and is approximately 30 microns at its widest point. It consists of large, irregularly shaped, lightly staining cells. Food vacuoles or ingested material were not observed.

Paired, dendritic vitellaria fill most of the ventrolateral spaces in the middle one-half of the body. These structures extend anteriorly to the level of the testes and posteriorly to the level of the cement glands (Fig. 1). In the distal ends of the branches are primordial cells. These primordia give rise to amber colored yolk cells which travel toward the midline and the collecting ducts. The yolk cells pass from each side into 2–5 collecting ducts which unite at the midline immediately dorsal to the egg capsule at the mouth of the ovovitelline duct.

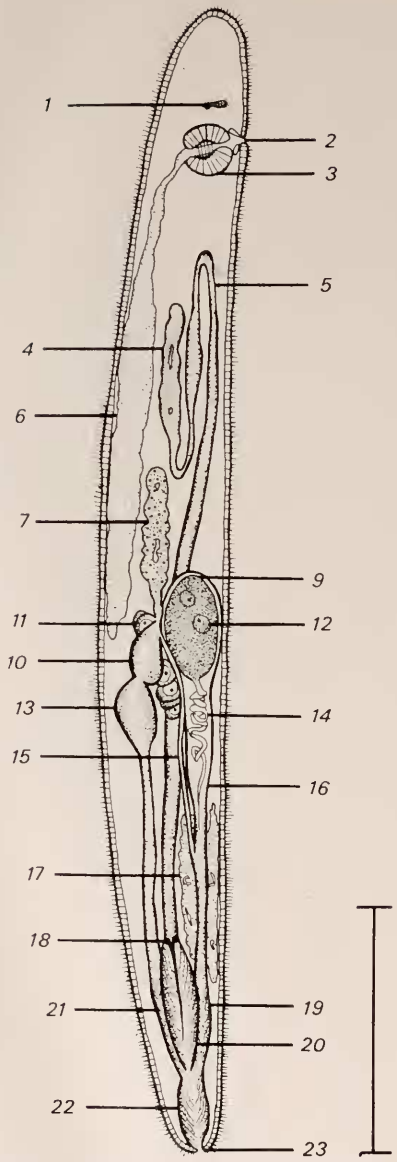

FIGURE 2. Sagittal section of *S. dendrastrorum*. Semidiagrammatic composite drawing. Scale bar equals 200 microns. Legend the same as in Figure 1.

The ovaries are single branches that arise at the mouth of the ovovitelline duct and extend posterolateral to the region of the cement glands (Fig. 1). The distal ends of the ovaries are sometimes lobed. Ovaries occur singly and were only seen to occur laterally in a cis relationship to a single testes. Stunkard and Corliss (1951) reported the occurrence of paired ovaries from the two specimens they

examined. From my work it is clear that the existence of paired ovaries is not the usual case, as only 3 out of 25 specimens examined exhibited this condition. An ovary branch consists of a chain of ova, which are proliferated from primordial germ cells at the distal end. Mature ova are approximately 16 microns in diameter. The nuclei of these cells measure approximately 2 microns in diameter.

For inclusion in the genus *Syndesmis* Silliman, 1881, a specimen must possess paired ovaries (Stunkard and Corliss, 1951). Based on my findings, *S. dendrastrorum* must be considered an exception. While *S. dendrastrorum* has been seen to occasionally possess paired ovaries, these clearly represent minority cases. When paired ovaries have been observed, the animals also possess paired testes.

The presence of the large egg capsule in mature worms obscures and distorts the reproductive organs in many of the preparations thus making accurate observation of these structures difficult. Such a difficulty was encountered by Stunkard and Corliss (1951), which resulted in their omission of any detailed study of the reproductive organs. In addition to encountering distortion from the egg capsule in this study, I found that the ducts of the genital antrum were crowded together in such a way that they were difficult to resolve. The density of the stain that accumulated in the cement glands also hampered observation. Fortunately, several of the sections prepared in this study were exceptionally fine, enabling accurate observations upon which is based the following account.

The seminal receptacle is oval, about 22 microns at its widest point, and arises at the mouth of the ovovitteline duct (Figs. 1 and 2). Its presence is obscured in whole mounts by the position of the large egg capsule. Immediately posterior and confluent to the seminal receptacle is the seminal bursa, which measures approximately 20 microns across (Figs. 1 and 2). Arising out of the posterior end of the seminal bursa is the bursal canal, a tubular structure about 26 microns long and 10 microns in diameter. It proceeds posteriorly to the posterior limit of the cement glands, where it becomes widened and possesses cilia-like projections about 6 microns long extending from the walls into the lumen (Figs. 1 and 2). The posterior end of the bursal canal then narrows again, opening dorsally into the anterior end of the common genital antrum.

The common genital antrum is an oval structure which measures about 26 microns in diameter and possesses cilia about 10 microns long that extend into its lumen. While possessing the bursal canal as a dorsal-anterior extension, the common genital antrum is also joined laterally at its anterior end by the male antrum, and medially by the female antrum (Figs. 1 and 2). The common genital pore opens ventrally at the posterior end of the worm.

The female antrum is ovoid, and measures about 12 microns in diameter. Like the bursal canal, it also possesses cilia that extend posteriorly into its lumen. The female antrum extends anteriorly and gives rise to the uterus. In the region of the cement glands the uterus receives the ovovitelline duct as a dorsal diverticulum (Figs. 1 and 2). From this junction the ovovitelline duct extends anteriorly along the midline to the union of the vitellaria, ovaries, and seminal receptacle. The ovovitelline duct is approximately 6 microns wide and is capable of expansion to allow yolk cells from the vitellaria and ova to pass into the uterus. This arrangement is typical of the family Umagillidae.

A criterion for inclusion in the subfamily Umagillinae is that the "ovovitelline duct enters common genital antrum ventral or anterior to the common sperm duct" (Stunkard and Corliss, 1951, page 331). I have found that *Syndesmis dendrastrorum* does not exhibit this characteristic. On the contrary, in *S. dendrastrorum* the ovovitelline duct enters the uterus anterior to the female antrum. *Syndesmis franciscana,* another member of the subfamily, also does not meet these requirements. Lehman (1946) reports that the ovovitelline duct enters the anterior end of the female antrum.

The uterus extends anteriorly along the ventral surface to the middle of the body. At this point the anterior end of the uterus is enlarged and encloses the oval, amber-colored egg capsule (Figs. 1 and 2). The cuticularized egg capsule encloses a mass of yolk cells and one to four ova. In mature worms this egg capsule averages 98 by 55 microns. The posterior end of the capsule is elongated and narrowed, forming a whip. This structure is approximately 10 microns in diameter, and is coiled in the region of the uterus just posterior to the egg capsule (Figs. 1 and 2). The cuticularized egg capsule and whip stain red with Mallory's triple stain and Harris' hematoxylin.

The lobed structures which lie in the ventrolateral spaces of the body on each side of the uterus consitute the cement glands (Fig. 1). Small ducts from these glands enter the uterus and extend from the enlarged portion which contains the whip, posteriorly to the junction of the female antrum. It is probable that secretions from these glands are used in fastening the egg capsule to the substrate when it is expelled (Lehman, 1946).

Lying in the anterior region of the body between the pharynx and the vitellaria are the testes (Figs. 1 and 2). In mature worms these male structures measure on the average 195 microns long and 93 microns wide. The testes consist of closely packed lobes which communicate with the sperm duct through many smaller ducts as in *S. franciscana* (Lehman, 1946). These small ducts unite in an enlarged portion of the sperm duct. The sperm duct continues anteriorly towards the pharynx doubling back on itself about two-thirds of the way between the egg capsule and the pharynx. Continuing posteriorly, the sperm duct coils around the egg capsule through the parenchyma uniting with the base of the penis in the posterior region of the cement glands (Figs. 1 and 2). The sperm duct is approximately 24 microns in diameter and contains many longitudinal and circular muscle fibers. The testes occur singly and in a lateral cis relationship to the single ovary. Although possession of paired testes is a criteria for inclusion in the subfamily Umagillinae as set forth by Stunkard and Corliss (1951), exclusion from the subfamily is unnecessary, as a number of *S. dendrastrorum* have been observed to possess two of these organs.

The penis is a cuticular tube approximately 74 microns long and 2 microns thick. The base of the penis is a cuticularized collar which unites the sperm duct with the male antrum (Figs. 1 and 2). These cuticularized structures, like the egg capsule, also stain red with Mallory's triple stain and Harris' hematoxylin. The penis is sheathed by a large male antrum, measuring 30 microns in diameter at its widest point. The male antrum extends from the cuticular collar of the penis past the tip of the copulatory organ, entering the common genital antrum slightly laterally (Figs. 1 and 2). The walls of the lumen of the male antrum are heavily ciliated. Individual cilia measure about 20 microns long. Longitudinal muscle

fibers in the walls of the male antrum and the sperm duct unite with the cuticularized collar of the penis. These fibers function as protractors and retractors of the penis as reported for *S. franciscana* (Lehman, 1946).

When my description and illustrations are compared with those originally presented by Stunkard and Corliss (1951), it might be inferred that different species are being described. However, the organisms described inhabit the same host, and the location and form of the vitellaria, the egg capsule and some of the other distinguishing characteristics presented in the two descriptions are similar. In addition, I have examined the type specimen deposited by Stunkard and Corliss, and have concluded that the differences are attributable to their contorted specimen.

During the dissection and examination of the hosts, I occasionally encountered small ciliated organisms. Closer examination of these forms revealed them to be juvenile stages of *S. dendrastrorum*. This is the first known account of juvenile stages in the entire family Umagillidae. The juveniles of *S. dendrastrorum* are opaque white, possessing the same shape as the adults. They vary from 0.28 mm to 0.77 mm in length and from 0.17 mm to 0.28 mm in width, depending on the stage of development. The youngest specimens, as judged by their small size, possess only a digestive system. In these young organisms a doliiform pharynx is present which occurs posteriorly, one-fourth of the distance from the anterior end. Unlike the adults, the intestine of the juvenile stages is clearly visible and quite large, filling most of the body posterior to the pharynx. As growth continues, the intestine of the worms becomes less prominent, probably as a result of differential growth, and occupies a smaller portion of the body. The vitellaria develops from a group of cells in the middle of the body. These structures, the first visible sex organs, branch laterally both in an anterior and posterior direction. By the time they have reached one-half their full size, ducts of the reproductive system appear in the posterior portion of the body. Following the development of the vitellaria, the testes, ovaries, and cement glands appear and the organism grows to become a mature adult. A preparation of several of these juvenile stages along with specimens of several representative adults has been deposited in the National Parasite Collection, United States Department of Agriculture, Beltsville, Maryland (Collection No. 72536).

From my work many exceptions to the criteria proposed by Stunkard and Corliss (1951) for the subfamily Umagillinae have become apparent. On this basis, I feel that further revision of the subfamily is warranted. In addition, there is no published evidence that members of the Umagillidae are parasitic *sensu stricto;* my observations on *S. dendrastrorum* indicate that these organisms are probably innocuous endocommensals.

I should like to thank Dr. Rosevelt L. Pardy, who guided me throughout this study.

Summary

1. *Syndesmis dendrastrorum* occurs in the intestine of the sand dollar *Dendraster excentricus.*

2. A detailed redescription of *S. dendrastrorum* is presented, clarifying the internal relationships of the reproductive organs.

3. The most distinguishing characteristics of this species include single ovaries and testes occurring in a cis relationship, and a large male antrum.

4. Juvenile stages of *S. dendrastrorum* possess only a digestive system and lack reproductive organs.

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